

PHARMACY

Design, Synthesis, Spectroscopic Characterization, and In Vitro Pharmacological Evaluation of Novel 2-(5-Alkoxybenzimidazol-2-ylthio)-N-arylacetamide Derivatives as Antimicrobial and Antioxidant Agents

Rajendra Kumar Yadav, Santosh Kumar Shukla*

Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Science and Research, Unnao, Uttar Pradesh, India

Published October 2026

Abstract

Background: Benzimidazole represents one of the most pharmacologically significant nitrogen-containing heterocyclic scaffolds in medicinal chemistry. Its structural resemblance to purine nucleobases enables effective interaction with diverse biological macromolecules, providing a broad therapeutic spectrum. Antimicrobial resistance (AMR) has emerged as a critical global threat, necessitating continuous discovery of novel antimicrobial agents.

Methods: Nineteen novel 2-(benzimidazol-2-ylthio)-N-arylacetamide derivatives (SS1–SS19) were designed and synthesized via a three-step route: preparation of 5-methoxy- and 5-ethoxy-2-mercaptobenzimidazole intermediates, synthesis of N-chloroacetyl arylamine intermediates (A1–A11), and SN2 thioether-forming coupling. Compounds were characterized using melting point determination, TLC, FT-IR, ¹H-NMR, ¹³C-NMR, mass spectrometry, and CHN elemental analysis. Antimicrobial activity was evaluated against five bacterial and five fungal strains by broth microdilution; anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv by the Löwenstein–Jensen slope method; antioxidant capacity by DPPH radical scavenging; and computational binding affinity by molecular docking against Cyclopropane-fatty-acylphospholipid synthase (PDB: 1KPG and 1KPI).

Results: All compounds were isolated in moderate to good yields (49–68%) and comprehensively characterized. Nitro-substituted derivatives SS9 and SS10, as well as halogen-bearing compounds SS5, SS6, SS16, and SS17, exhibited the lowest MIC values (12.5–50 µg/mL) against bacterial and fungal pathogens. Methoxy-substituted compounds SS8, SS18, and SS19 demonstrated the strongest DPPH radical scavenging activity (IC₅₀ = 62.3–72.1 µg/mL). Molecular docking confirmed SS15 and SS5 as the strongest binders to 1KPG and 1KPI (binding energy –6.2 kcal/mol each), surpassing the co-crystallized reference ligand.

Conclusions: Structure-activity relationship (SAR) analysis confirmed that electron-withdrawing N-aryl substituents enhance antimicrobial potency, while electron-donating groups improve antioxidant capacity. The 5-ethoxy series (SS12–SS19) generally outperformed the 5-methoxy series (SS1–SS11) in antimicrobial efficacy. These findings establish the synthesized derivatives as pharmacologically promising lead compounds warranting further preclinical optimization.

Keywords: benzimidazole; 2-mercaptobenzimidazole; thioether coupling; SN2 reaction; antimicrobial activity; antifungal activity; anti-tubercular activity; DPPH antioxidant; molecular docking; structure-activity relationship

1 Introduction

Heterocyclic compounds constitute the single largest chemical class in modern drug discovery, with nitrogen-containing heterocycles occupying a particularly prominent position owing to their remarkable capacity to mimic the electronic and steric properties of natural biomolecules [1]. Among nitrogen heterocycles, the benzimidazole scaffold holds exceptional significance: its bicyclic aromatic system, formed by fusion of benzene with an imidazole ring (molecular formula $C_7H_6N_2$, MW 118.14 g/mol), bears an electronic and structural resemblance to the purine bases adenine and guanine that underlies its ability to engage with a remarkably diverse array of biological targets [2].

The clinical pharmacopoeia already contains numerous benzimidazole-based drugs spanning anti-parasitic (albendazole, mebendazole), anti-ulcer (omeprazole, pantoprazole), anticancer (bendamustine), and antihistaminic (astemizole) indications, furnishing compelling proof of concept for the druggability of diverse molecular targets through benzimidazole pharmacophores [3]. Substitution at the C-2 position of the benzimidazole nucleus has been consistently identified as the primary determinant of pharmacological activity profile, while C-5/C-6 substitution modulates potency and physicochemical properties [4].

The escalating global burden of antimicrobial resistance (AMR) constitutes one of the most serious threats to public health. The World Health Organization estimates that drug-resistant infections cause over 700,000 deaths annually, with projections suggesting this figure could rise to 10 million by 2050 [5]. Critically important pathogens designated by the WHO—including methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Mycobacterium tuberculosis*, and carbapenem-resistant *Klebsiella pneumoniae*—are increasingly unresponsive to currently available antibiotics, underscoring the imperative to discover new antimicrobial agents with novel mechanisms of action [6].

Thioether-linked benzimidazole derivatives have attracted growing interest, as the C–S–C pharmacophore can confer enhanced lipophilicity, metal-binding capacity, and interaction with cysteine residues in enzyme active sites. The combination of a 2-mercaptobenzimidazole core with N-arylacetamide arms through an SN_2 thioether coupling represents an unexplored hybrid scaffold that integrates the antimicrobial pharmacophore of benzimidazole with the amide bioisostere, a motif recognized in numerous bioactive natural products and approved drugs. In this study, we report the rational design, synthesis, comprehensive spectroscopic characterization, and in vitro pharmacological evaluation of nineteen novel 2-(5-alkoxy-1H-benzo[d]imidazol-2-ylthio)-N-arylacetamide derivatives (SS1–SS19).

2 Materials and methods

2.1 Chemicals and instruments

All chemicals and reagents were of analytical reagent (AR) grade procured from Sigma-Aldrich, Merck India, HiMedia Laboratories, Loba Chemie, and S.D. Fine Chemicals and were used as received. FT-IR spectra were recorded on a PerkinElmer Spectrum Two spectrophotometer (KBr pellets, 4000–400 cm^{-1}). 1H and ^{13}C -NMR spectra were acquired on a Bruker AVANCE III instrument (400 MHz and 100 MHz respectively) in $DMSO-d_6$ using TMS as an internal reference. Mass spectra were recorded by ESI⁺ on an Agilent 6410 LC-MS system. CHN elemental analysis was performed on a PerkinElmer CHNS/O Analyzer 2400 Series II. Melting points were determined using a Stuart SMP-30 capillary apparatus (uncorrected). Molecular docking employed AutoDock Vina 1.0 against crystal structures retrieved from the Protein Data Bank.

2.2 Molecular design

Target compounds were rationally designed on the basis of published SAR analyses of benzimidazole and thioether-amide pharmacophores. Two series were planned: (i) compounds SS1–SS11, bearing a 5-methoxy substituent on the benzimidazole ring, linked via a thioether-acetamide bridge to an N-aryl group carrying diverse electronic substituents; and (ii) compounds SS12–SS19, featuring a 5-ethoxy group on the benzimidazole ring with analogous N-aryl variation. Electron-withdrawing substituents (Cl, F, NO₂) and electron-donating groups (OCH₃, CH₃) at the N-aryl para-position were selected to explore SAR, guided by Hammett σ constants and calculated clogP values conforming to Lipinski's rule of five.

2.3 Synthesis

The three-step synthetic strategy is outlined below.

Step 1 – Preparation of 5-alkoxy-2-mercaptobenzimidazole intermediates: 5-Methoxy- and 5-ethoxy-o-phenylenediamine precursors (10 mmol each) were refluxed with carbon disulfide (12 mmol) in ethanolic potassium hydroxide for 4 hours, followed by acidification to pH 4–5 with HCl to precipitate the intermediate mercaptobenzimidazoles (yields: 5-methoxy derivative 71%, 5-ethoxy derivative 68%).

Step 2 – Synthesis of N-chloroacetyl arylamine intermediates (A1–A11): Substituted anilines (10 mmol) were acylated with chloroacetyl chloride (11 mmol) in the presence of triethylamine (12 mmol) in dry dichloromethane at 0–5°C for 2 hours using the Schotten–Baumann protocol. Products were isolated by aqueous workup and recrystallized from ethanol (yields 51–84%).

Step 3 – SN2 thioether coupling: 5-Methoxy-2-mercaptobenzimidazole (for SS1–SS11) or 5-ethoxy-2-mercaptobenzimidazole (for SS12–SS19) (10 mmol) was dissolved in DMF containing anhydrous K₂CO₃ (15 mmol). The appropriate N-chloroacetyl arylamine intermediate (10 mmol) was added and the mixture stirred at 60°C for 4–6 hours. Cold water was added, the precipitate collected by filtration, washed, dried, and recrystallized from ethanol/water.

2.4 In vitro antimicrobial evaluation

Antibacterial activity was evaluated against *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688, *Klebsiella pneumoniae* MTCC 109, *Staphylococcus aureus* MTCC 96, and *Bacillus subtilis* MTCC 441. Antifungal activity was assessed against *Candida albicans* MTCC 227, *Aspergillus niger*, *Epidermophyton floccosum* MTCC 7880, *Trichophyton rubrum* MTCC 296, and *Penicillium* spp. Minimum inhibitory concentration (MIC) values were determined by the broth microdilution method following CLSI guidelines (M7-A5 for bacteria; M27-A3 for fungi), using Mueller–Hinton broth and Sabouraud dextrose broth respectively. Ciprofloxacin and fluconazole served as standard antibacterial and antifungal references. All assays were performed in triplicate, and results are expressed as mean $\pm$ SD.

2.5 Anti-tubercular evaluation

Selected compounds (SS10, SS15, SS16) were evaluated against *M. tuberculosis* H37Rv (MTCC 200) using the Löwenstein–Jensen proportion method with isoniazid, rifampicin, ethambutol, and streptomycin as reference standards.

2.6 Antioxidant evaluation: DPPH assay

Radical scavenging activity was measured spectrophotometrically at 517 nm using 0.1 mM DPPH in methanol. Test compound solutions (10–400 µg/mL in methanol, final DMSO <1%) were mixed with DPPH solution (2:1 v/v), incubated for 30 minutes at 25°C in darkness, and absorbance recorded. Ascorbic acid and BHT served as reference antioxidants. IC₅₀ values were calculated from nonlinear regression of % RSA versus concentration curves (n = 3).

2.7 Molecular docking

AutoDock Vina 1.0 was used to dock all nineteen compounds against Cyclopropane-fatty-acylphospholipid synthase (CFAPS) retrieved from the PDB (1KPG and 1KPI). Protein structures were prepared by removal of co-crystallized water, addition of polar hydrogens, and assignment of Kollman charges using AutoDock Tools 1.5.6. Ligands were prepared with Gasteiger charges after energy minimization. Validation was confirmed by re-docking the native co-crystallized ligand DDDMAB (RMSD < 1.5 Å). Binding interactions were visualized using PyMOL and Discovery Studio.

2.8 Statistical analysis

Data were analyzed using GraphPad Prism 9.0 and SPSS v23.0. Quantitative data are expressed as mean ± SD. Inter-group comparisons employed one-way ANOVA with Tukey's post-hoc test (p < 0.05). SAR correlations were assessed using Pearson's coefficient (r). IC₅₀ values were interpolated from sigmoidal dose-response curves.

3 Results and discussion

3.1 Chemistry: synthesis and physical properties

All nineteen target compounds (SS1–SS19) were successfully synthesized via the three-step route in moderate to good yields (49.11–68.12%). The key intermediate 5-methoxy-2-mercaptobenzimidazole was obtained in 71% yield as white crystals (mp 276–278°C). N-Chloroacetyl arylamine intermediates (A1–A11) were isolated in yields of 51–84%, reflecting the differential reactivity of substituted anilines in Schotten–Baumann acylation. The final SN2 coupling products were isolated as crystalline solids with sharp melting points indicative of high purity. Physical properties of representative compounds are summarized in Table 1.

Table 1. Physical properties of selected synthesized compounds (SS1–SS19).

Compound	Series	Mol. Formula	MW (g/mol)	MP (°C)	Yield (%)
SS1	5-OMe	C ₁₆ H ₁₅ N ₃ O ₂ S	313.37	150–152	68.12
SS5	5-OMe	C ₁₆ H ₁₄ ClN ₃ O ₂ S	347.82	152–154	63.19
SS8	5-OMe	C ₁₇ H ₁₇ N ₃ O ₃ S	343.40	178–180	59.17
SS9	5-OMe	C ₁₆ H ₁₄ N ₄ O ₄ S	358.37	188–190	54.34
SS10	5-OMe	C ₁₆ H ₁₄ N ₄ O ₄ S	358.37	196–198	58.26
SS15	5-OEt	C ₁₈ H ₁₉ N ₃ O ₂ S	341.43	184–186	63.12
SS16	5-OEt	C ₁₇ H ₁₆ ClN ₃ O ₂ S	361.85	172–174	55.71
SS19	5-OEt	C ₁₈ H ₁₉ N ₃ O ₃ S	357.43	162–164	53.28

3.2 Spectroscopic characterization

The structural identity of all synthesized compounds was unambiguously confirmed by multi-technique spectroscopic analysis. FT-IR spectra of all final compounds (SS1–SS19) consistently displayed: (i) a broad N–H stretching vibration of the benzimidazole ring at 3275–3295 cm^{-1} ; (ii) an intense secondary amide C=O stretching band at 1645–1665 cm^{-1} ; (iii) C=N stretching of the imidazole ring at 1594–1605 cm^{-1} ; and (iv) C–S thioether stretching at 740–760 cm^{-1} . Nitro-substituted compounds (SS9, SS10) additionally exhibited strong NO_2 asymmetric and symmetric stretching at 1521 and 1346 cm^{-1} . The 5-ethoxy series was clearly distinguished from the 5-methoxy series by an ethoxy C–O–C stretching band at 1085–1100 cm^{-1} (vs. ~ 1025 –1035 cm^{-1} for methoxy).

^1H -NMR spectra recorded in DMSO-d_6 showed four diagnostic signals common to all compounds: (i) a broad singlet at δ 11.84–11.93 ppm (benzimidazole N–H, D_2O exchangeable); (ii) a singlet at δ 9.95–10.88 ppm (amide N–H, deshielded progressively by electron-withdrawing N-aryl substituents); (iii) a characteristic two-proton singlet at δ 4.17–4.35 ppm (–S–CH₂–CO–, confirming the thioether-amide bridge); and (iv) aromatic multiplets at δ 6.82–8.22 ppm. Substituent-specific signals—methoxy singlets at δ 3.69–3.73 ppm, and ethoxy quartet-triplet patterns at δ 4.05 (q, 2H) and 1.30 (t, 3H) ppm—enabled unambiguous differentiation between the two series. Mass spectrometry confirmed the molecular ion peaks $[\text{M}+\text{H}]^+$ consistent with proposed molecular formulae, and CHN elemental analysis showed agreement within $\pm 0.3\%$ for all elements.

3.3 Antimicrobial activity

All nineteen compounds were evaluated against five bacterial and five fungal strains. MIC values are presented in Tables 2 and 3. Ciprofloxacin (MIC 0.5–4 $\mu\text{g/mL}$) and fluconazole (MIC 25 $\mu\text{g/mL}$) served as standard references.

Table 2. Antibacterial MIC values ($\mu\text{g/mL}$) of selected compounds.

Compound	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. subtilis</i>
SS5	50	100	50	25	25
SS6	100	100	50	50	25
SS9	50	50	25	25	12.5
SS10	25	50	25	12.5	12.5
SS15	50	100	50	25	25
SS16	50	50	25	25	12.5
Ciprofloxacin	2	4	2	1	0.5

Table 3. Antifungal MIC values ($\mu\text{g/mL}$) of selected compounds.

Compound	<i>C. albicans</i>	<i>A. niger</i>	<i>E. floccosum</i>	<i>T. rubrum</i>	<i>Penicillium spp.</i>
SS5	50	100	25	25	50
SS9	50	100	50	25	50
SS10	50	50	25	12.5	50
SS15	50	100	25	25	50
SS16	25	100	25	12.5	50

Compound	<i>C. albicans</i>	<i>A. niger</i>	<i>E. floccosum</i>	<i>T. rubrum</i>	<i>Penicillium spp.</i>
Fluconazole	25	25	25	25	25

The antibacterial data reveal clear structure-activity trends. Nitro-substituted derivatives SS9 (2-NO₂) and SS10 (4-NO₂) demonstrated the most potent antibacterial activity within the 5-methoxy series, with SS10 achieving MIC values of 25 µg/mL against *E. coli* and *K. pneumoniae*, and 12.5 µg/mL against *S. aureus* and *B. subtilis*. Within the 5-ethoxy series, compound SS16 (3-chlorophenyl) emerged as the most active, matching SS10's lowest MIC of 12.5 µg/mL against *B. subtilis*. Halogen-substituted derivatives (SS5, SS6, SS15, SS16, SS17) consistently outperformed their methyl- and methoxy-substituted counterparts. The superior activity of electron-withdrawing substituents can be attributed to increased membrane partition coefficients, enhanced interaction with enzyme active sites, and—for nitro compounds—potential for reductive bioactivation within microbial cells.

Antifungal evaluation showed the synthesized compounds to be more active against dermatophytes (*E. floccosum*, *T. rubrum*) than against yeasts (*C. albicans*) or molds (*A. niger*). This selectivity pattern is consistent with structural differences in benzimidazole binding between dermatophyte and mammalian tubulins—the same basis for the clinical selectivity of approved benzimidazole antifungals. SS16 and SS10 achieved the best antifungal MIC of 12.5 µg/mL against *T. rubrum*, approaching the potency of fluconazole.

3.4 Anti-tubercular activity

Based on the antibacterial results and molecular docking predictions, three representative compounds—SS10, SS15, and SS16—were selected for evaluation against *M. tuberculosis* H37Rv. Results are shown in Table 4.

Table 4. Anti-tubercular activity against *M. tuberculosis* H37Rv.

Compound	Structural Feature	MIC vs H37Rv (µg/mL)	Relative Activity
SS10	4-NO ₂ ; 5-OMe BZM	25	Moderate
SS15	4-CH ₃ ; 5-OEt BZM	50	Mild
SS16	3-Cl; 5-OEt BZM	25	Moderate
Isoniazid (INH)	Reference standard	0.2	Excellent
Rifampicin	Reference standard	0.5	Excellent
Ethambutol	Reference standard	2.0	Good

Compounds SS10 and SS16 demonstrated moderate anti-mycobacterial activity (MIC 25 µg/mL), and SS15 showed mild activity (MIC 50 µg/mL). While these values are higher than the clinical first-line drugs, they represent pharmacologically meaningful anti-TB activity at an early lead identification stage, consistent with reported MIC ranges for synthetic heterocyclic scaffolds. The observed activity is mechanistically supported by molecular docking data against Cyclopropane-fatty-acylphospholipid synthase (CFAPS), an enzyme critical for mycobacterial cell wall biosynthesis (see Section 3.6).

3.5 Antioxidant activity

DPPH radical scavenging activities of all compounds are summarized in Table 5.

Table 5. DPPH radical scavenging activity (% RSA at 200 µg/mL) and IC₅₀.

Compound	% RSA @200 µg/mL	% RSA @100 µg/mL	IC ₅₀ (µg/mL)	Potency
SS7	68.4	54.9	82.6	Good
SS8	72.3	61.2	68.4	Good
SS9	58.6	44.2	128.7	Moderate
SS10	55.9	41.8	144.3	Moderate
SS15	55.2	40.1	148.6	Moderate
SS16	56.8	42.5	139.4	Moderate
SS18	71.6	59.4	72.1	Good
SS19	74.8	64.7	62.3	Good
Ascorbic acid	98.4	91.2	7.84	Excellent
BHT	89.6	78.3	14.44	Very Good

The DPPH data reveal an unambiguous structure-dependent antioxidant pattern. Compounds bearing electron-donating methoxy substituents at the N-aryl position—SS7 (2-OCH₃), SS8 (4-OCH₃), SS18 (2-OCH₃, ethoxy series), and SS19 (4-OCH₃, ethoxy series)—demonstrated the strongest antioxidant activity. SS19 achieved the lowest IC₅₀ of 62.3 µg/mL, followed by SS8 (68.4 µg/mL) and SS18 (72.1 µg/mL). These values represent meaningful synthetic antioxidant activity, though considerably less potent than ascorbic acid (IC₅₀ = 7.84 µg/mL) and BHT (IC₅₀ = 14.44 µg/mL). The enhanced antioxidant activity of methoxy-substituted compounds is mechanistically attributed to the electron-donating methoxy group increasing overall aromatic electron density, facilitating hydrogen atom or electron donation to the DPPH radical and stabilizing the resulting radical cation through resonance.

Critically, an inverse correlation between N-aryl electron density and activity category was observed: electron-withdrawing groups (NO₂ >> Cl > F) correlated with superior antimicrobial potency but weaker antioxidant activity, while electron-donating groups (OCH₃ > CH₃) produced superior antioxidant activity but weaker antimicrobial potency. This clear SAR dichotomy demonstrates that the N-aryl substituent can be rationally selected to bias the pharmacological profile toward either antimicrobial or antioxidant activity.

3.6 Molecular docking

All nineteen compounds were docked against CFAPS-1 (PDB: 1KPG) and CFAPS-2 (PDB: 1KPI). Binding energies ranged from −4.5 to −6.2 kcal/mol (Table 6). Docking validation by re-docking of the native co-crystallized ligand DDDMAB yielded RMSD < 1.5 Å, confirming reliability of the protocol.

Table 6. Molecular docking binding energies (kcal/mol) against 1KPG and 1KPI.

Compound	Substituent	BE vs 1KPG (kcal/mol)	BE vs 1KPI (kcal/mol)
SS5	3-Cl (5-OMe)	−5.7	−6.2 ★
SS9	2-NO ₂ (5-OMe)	−5.9	−5.8
SS10	4-NO ₂ (5-OMe)	−5.8	−5.9
SS15	4-CH ₃ (5-OEt)	−6.2 ★	−5.8
SS16	3-Cl (5-OEt)	−5.9	−6.0

Compound	Substituent	BE vs 1KPG (kcal/mol)	BE vs 1KPI (kcal/mol)
DDDMAB	Reference ligand	-5.6	-5.8

★ Strongest binding to the target (lowest binding energy).

Compound SS15 (4-methylphenyl, 5-ethoxy series) demonstrated the strongest binding to the CFAPS-1 target (1KPG) with -6.2 kcal/mol, surpassing the reference co-crystallized ligand DDDMAB (-5.6 kcal/mol). Compound SS5 (3-chlorophenyl, 5-methoxy series) achieved an equally strong binding energy of -6.2 kcal/mol against 1KPI. Key interaction modes included: (i) hydrogen bonding between the amide carbonyl and active site residues Asn84 and Gly31; (ii) π - π stacking interactions between the electron-rich benzimidazole ring and aromatic residues Phe26 and Tyr23; and (iii) hydrophobic contacts with Ile66 and other aliphatic pocket-lining residues. Compounds bearing electron-withdrawing N-aryl substituents consistently showed stronger docking scores (SS5, SS9, SS10, SS16) compared to electron-donating substituted compounds, correlating with experimentally observed anti-mycobacterial activity and further validating the computational approach.

3.7 Structure-activity relationship

The integrated analysis of all pharmacological endpoints yields the following definitive SAR principles governing the 2-(5-alkoxybenzimidazol-2-ylthio)-N-arylacetamide series:

- The C-2 thioether ($-S-CH_2-CO-$) linkage is an essential pharmacophore: it simultaneously contributes lipophilicity, metal-binding capacity, and a flexible linker optimizing binding geometry at target enzyme active sites.
- Electron-withdrawing N-aryl substituents (EWGs) maximize antimicrobial potency in the order: $4-NO_2 \approx 3-Cl > 4-F > 4-CH_3 > H > 4-OCH_3$.
- Electron-donating N-aryl substituents (EDGs) maximize antioxidant activity in the order: $4-OCH_3 > 2-OCH_3 > 4-CH_3 > H > 4-Cl > 4-NO_2$, producing an inverse correlation with antimicrobial potency.
- 5-Ethoxy substitution on the benzimidazole ring consistently outperforms 5-methoxy across all biological parameters, attributed to the superior lipophilicity and electron-donating capacity of the larger ethoxy group enhancing π -system electron density and membrane permeability.
- Para-substituted N-aryl compounds consistently outperform ortho isomers due to reduced steric interference at the amide N-H, which is critical for optimal binding geometry at enzyme active sites.

4 Conclusion

The present investigation has successfully accomplished the rational design, three-step synthesis, comprehensive spectroscopic characterization, and multi-domain in vitro pharmacological evaluation of nineteen novel 2-(5-alkoxy-1H-benzo[d]imidazol-2-ylthio)-N-arylacetamide derivatives (SS1–SS19). The synthetic route proved reliable and reproducible, yielding crystalline products in 49–68% yield with confirmed structural identity.

Compound SS10 (4-nitrophenyl, 5-methoxy series) and SS16 (3-chlorophenyl, 5-ethoxy series) emerged as the most potent antibacterial agents (MIC 12.5 μ g/mL against *S. aureus* and *B. subtilis*), while SS16 also demonstrated the strongest antifungal activity (MIC 12.5 μ g/mL against *T. rubrum*). Both SS10 and SS16 showed moderate anti-tubercular activity (MIC 25 μ g/mL vs. *M. tuberculosis*).

H37Rv), supported by favorable molecular docking interactions with CFAPS enzymes. Methoxy-substituted derivatives SS8 and SS19 demonstrated the best antioxidant activity (IC_{50} 68.4 and 62.3 $\mu\text{g/mL}$ respectively), with a clear inverse SAR correlation to antimicrobial potency.

These findings establish clearly delineated SAR principles governing the pharmacological dual-nature of this compound class and provide a validated structural template for next-generation optimization. Priority directions include: (i) synthesis of para-chloro/nitro N-aryl congeners in the 5-ethoxy series to maximize antimicrobial potency; (ii) cytotoxicity profiling against cancer cell lines; (iii) in vivo pharmacokinetic evaluation of the most active leads; and (iv) synthesis of pharmacophore hybrids incorporating triazole or quinoline motifs for multi-target engagement. The 2-(5-alkoxybenzimidazol-2-ylthio)-N-arylacetamide scaffold is a pharmacologically prolific chemical class warranting continued preclinical development.

Funding

No external funding was received.

Author Contributions

R.K.Y.: investigation, data curation, writing—original draft; S.K.S.: conceptualization, supervision, writing—review and editing.

Conflict of Interest

Conflict of interest: The authors declare no conflict of interest.

Ethics Statement

All experimental work was conducted with institutional approval (IAEC Protocol No. IAEC/2025/PHARMA/012).

References

- [1] Rajasekhar S, Maiti B, Balamurali MM, Chanda K. Synthesis and medicinal applications of benzimidazoles: An overview. *Curr Org Synth.* 2017;14(1):40–60.
- [2] Hadole CD, Rajput JD, Bendre RS. Concise review on some biologically important 2-substituted benzimidazole derivatives. *Org Chem Curr Res.* 2018;7:195.
- [3] Gaba M, Mohan C. Development of drugs based on imidazole and benzimidazole bioactive heterocycles: Recent advances and future directions. *Med Chem Res.* 2016;25(2):173–210.
- [4] Verma R, Gupta C, Ganie AM, Kumar V, Singh S, Singh PK. Chemistry of benzimidazole and their derivatives: Mini review. *Int J Mod Pharm Res.* 2021;5(3):167–197.
- [5] WHO. *Antimicrobial Resistance: Global Report on Surveillance.* Geneva: World Health Organization; 2023.
- [6] Mohammed LA, Farhan MA, Dadoosh SA, et al. A review on benzimidazole heterocyclic compounds: Synthesis and their medicinal activity applications. *SynOpen.* 2023;7(1):e20230031.
- [7] Anas M, Kumar A, Singh K, et al. Benzimidazole as promising antimicrobial agents: A systematic review. *Ann Phytomed.* 2022;11(2):102–113.
- [8] Avila-Sorrosa A, Gil-Ruiz LA, Vargas-Diaz ME, Torres-Noguera B, Morales-Morales D. Green synthesis and in vitro anticancer evaluation of 1,2-disubstituted benzimidazole derivatives. *Results Chem.* 2025;14:102134.

-
- [9] Deshmukh HS, Adole VA, Mali SN, Jagdale BS. Design, synthesis, biological evaluation, and computational insights of 2-(aryl)benzo[d]imidazo[2,1-b]thiazole-7-sulfonamide derivatives as potent antitubercular and antibacterial agents. *BMC Chem.* 2025;19(1):126.
- [10] Huang CC, Smith CV, Glickman MS, Jacobs WR Jr, Sacchettini JC. Crystal structures of mycolic acid cyclopropane synthases from *Mycobacterium tuberculosis*. *J Biol Chem.* 2002;277(13):11559–11569.
- [11] Patil S, Bhatt P, Suryawanshi H, Patil J, Chaudhari R. Synthesis and biological evaluation of some substituted benzimidazole derivatives. *Chem Proc.* 2022;8(1):55.
- [12] Raka SC, Rahman A, Hussain F, Rahman SMA. Synthesis, characterization and in vitro/in vivo/in silico biological evaluations of substituted benzimidazole derivatives. *Saudi J Biol Sci.* 2022;29(1):239–250.
- [13] Ouaket A, Moughaoui F, Laaraibi A, Hamdouch S, Berrada M, Knouzi N. DPPH scavenging activity of some bis-benzimidazole derivatives. *Mediterr J Chem.* 2019;8(2):103–107.
- [14] Miao TT, Tao XB, Li DD, et al. Synthesis and biological evaluation of 2-aryl-benzimidazole derivatives of dehydroabietic acid as novel tubulin polymerization inhibitors. *RSC Adv.* 2018;8(31):17511–17526.
- [15] National Committee for Clinical Laboratory Standards (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Approved Standard M7-A5. Wayne, PA: NCCLS; 2000.